Nucleolus organizer region heteromorphism in patients with Down syndrome and their parents

N. A. Nazmy, S.M. Kotb, M.M. Mokhtar and S.R. Ismail

Abstract

The study aimed to evaluate the role of nucleolus organizer region (NOR) heteromorphism as an etiological factor for parental nondisjunction in Down syndrome by comparing 25 patients affected by Down syndrome, and their parents with a control group of 80 non-affected Egyptians. All parents had normal karyotypes. The average modal number per parent of Ag-positive NORs was significantly higher in parents than controls. A significant difference in the size of the double NOR variants (dNORs) was found. The mean maternal and paternal ages were significantly lower, with a significant increase in spontaneous abortions, for dNOR(+) couples compared with dNOR(−) couples.

L'hétéromorphisme de la région de l'organisateur nucléolaire chez les patients atteints du syndrome de Down

Cette étude visait à évaluer le rôle de l'hétéromorphisme de la région de l'organisateur nucléolaire comme facteur étiologique de la non-disjonction chez les parents dans le syndrome de Down en comparant 25 patients atteints du syndrome de Down et leurs parents avec un groupe de témoins composé de 80 Egyptiens non atteints. Tous les parents avaient un caryotype normal. Le nombre modal moyen par parent de régions de l'organisateur nucléolaire pour lesquelles l'antigène était positif était considérablement plus élevé chez les parents que chez les témoins. Une différence significative de la taille des variantes de la double région de l'organisateur nucléolaire a été trouvée. L'âge moyen de la mère et du père était considérablement moins élevé, avec une augmentation significative des avortements spontanés pour les couples ayant une région de l'organisateur nucléolaire à antigène positif par rapport à ceux dont l'antigène était négatif.

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Introduction

Down syndrome results from trisomy 21, the presence of an extra chromosome 21, either free or as a part of Robertsonian fusion [1]. About 90–95% of the time the nondisjunction event leading to trisomy occurs in the mother, with the error being predominantly in meiosis I [2]. The short arms of human acrocentric chromosomes are the sites of heteromorphisms, detectable by a silver staining technique specifically for nucleolus organizer regions (NORs) [3]. Heteromorphisms of these regions include variation in the pattern and duplication of NORs [4]. This has been proposed as a risk factor for nondisjunction of acrocentric and sex chromosomes [5].

The increased risk of recurrence of Down syndrome (DS) for couples who have previously had a child affected by trisomy 21 may be attributed to a parent carrying a dNOR variant [5]. Analysis of NOR heteromorphism can be of value in the management of DS. When present in a parent the dNOR variant may be an important new indicator in antenatal monitoring of at-risk pregnancies [5].

The study aimed to evaluate the possible role of NOR heteromorphism as an etiological factor for parental nondisjunction in DS.

The chromosome preparations were incubated in 50% silver nitrate at 37 °C for 12–72 hours, then in distilled water. Ammoniacal silver solution and 3% formalin solution (two drops of each) were placed in the slides. Staining intensity was monitored until a golden-yellow colour obtained. The slides were then rinsed in distilled water and banded with trypsin G-banding.

NOR analysis was carried out as follows: A NOR was taken to be Ag-positive if a distinct black stain could be recognized on the short arms of the acrocentric chromosomes. The number of Ag-stained NORs per metaphase was expressed as the modal number for each individual. The size of NORs in each individual was expressed in arbitrary units (1–5) using the short arm of chromosome 18 (18p) as the reference point. The presence of dNORs was recorded as dNOR(+) if the criteria for dNORs were fulfilled (elongated stalk; two distinct vertical areas of stain, separate or confluent; expression of two stained areas in more than 20% of cells scored). The results of NOR analysis were compared with a control group, which consisted of 80 normal Egyptian individuals (40 males and 40 females) [8].

Results

The age of the patients ranged from 8 days to 5.4 years with a mean of 1.3 ± 1.4 years. Male to female ratio was 2.1:1. Of the mothers, 32% were aged 35 years or older, while 8% of fathers were 45 years or older. The mean maternal age was 31 ± 6 years and mean paternal age 35 ± 6.29 years.

Cytogenetic studies

G-banding: All cases were standard trisomy 21. The parents had normal karyotypes. NOR stain: The presence, size and
morphology of NORs on the acrocentric chromosomes in DS patients and their parents were compared with the control group (Tables 1–4). The range of the modal number of Ag-positive NORs in the parents was 7–10, with an average of 8.06 ± 0.83, compared with 7.65 ± 0.98 in the controls — a statistically significant difference. The average modal number of Ag-positive NORs per patient was 9.04 ± 1.08, which was significantly higher than for the controls (Table 1). Variation in the size of Ag-positive NORs is shown in Table 2. A significant difference in the size of NORs was found between parents and controls ($\chi^2 = 17.13$, $P < 0.05$) and between patients and controls ($\chi^2 = 11.5$, $P < 0.05$).

There were five NOR variants on the acrocentric chromosomes distinguished (Figure 1):
1. absent
2. simple indistinct (siNOR)
3. simple (sNOR)
4. massive (mNOR)
5. double (dNOR).

The distribution of dNOR variants on different chromosomes in the parents, and their inheritance by the proband, are shown in Table 3. Of the parents, 16% were found to be carriers of a dNOR variant. Three DS patients (12%) had a dNOR variant — two males with dNOR on chromosomes 2 and 22, and one female with dNOR on chromosome 14. The three patients had inherited

<table>
<thead>
<tr>
<th>Table 1 Average modal number of Ag-positive NORs per individual in Down syndrome patients, their parents and the control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Down syndrome patients ($n = 25$)</td>
</tr>
<tr>
<td>Parents ($n = 50$)</td>
</tr>
<tr>
<td>Control ($n = 80$)</td>
</tr>
</tbody>
</table>

*statistically significant
$s = standard deviation$

<table>
<thead>
<tr>
<th>Table 2 Size distribution of Ag-positive NORs in Down syndrome patients, their parents and the control group</th>
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</thead>
<tbody>
<tr>
<td>Size of NORs</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Very large</td>
</tr>
<tr>
<td>Large</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>Small</td>
</tr>
<tr>
<td>Absent</td>
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<tr>
<td>Total</td>
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</table>

$\chi^2$ test versus controls 11.5* 17.13* |

*statistically significant
Figure 1 Silver stained metaphase showing Ag-positive NORs on only eight acrocentric chromosomes: a) a patient with Down syndrome; b) the patient’s mother

Table 3 Distribution of dNOR variant on different chromosomes in the parents of Down syndrome patients and their inheritance by the proband

<table>
<thead>
<tr>
<th>Family No.</th>
<th>dNOR(+) chromosome</th>
<th>No. of dNORs per 20 cells</th>
<th>dNOR(+) parent</th>
<th>Inheritance of dNOR(+) by proband</th>
<th>Parental age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>6</td>
<td>30</td>
<td>M</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>10</td>
<td>50</td>
<td>P</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>8</td>
<td>40</td>
<td>M</td>
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<td>5</td>
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<tr>
<td>6</td>
<td>15</td>
<td>12</td>
<td>60</td>
<td>M</td>
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<td>7</td>
<td>21</td>
<td>6</td>
<td>30</td>
<td>M</td>
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<tr>
<td>8</td>
<td>14</td>
<td>10</td>
<td>50</td>
<td>M</td>
<td>+</td>
</tr>
</tbody>
</table>

M = maternal  P = paternal

the dNOR variant from their mothers. The frequency of dNOR variants in the parents, DS patients and controls is shown in Table 4.

Comparison between dNOR(+) couples (8 of the 25) and dNOR(−) couples showed the mean maternal and paternal ages for dNOR(+) cases to be significantly lower than those for dNOR(−) (t = −2.5, P < 0.05; t = −2.6, P < 0.05). Parental consanguinity showed no statistically significant difference between dNOR(+) and dNOR(−) couples, (χ² = 0.64, P > 0.05). In one case, a family history of DS was found in a relative
of the mother; the mother was found to carry a dNOR variant. Increased frequency of first trimester spontaneous abortion was observed among the dNOR(+) couples (33.3%) as compared with dNOR(−) couples (7.4%). The difference was statistically significant ($\chi^2 = 4.27, P < 0.05$).

**Discussion**

Down syndrome has always been of special interest to human genetics because it has the highest incidence of chromosomal abnormalities at birth, and is the most common genetic cause of mental retardation [9]. The possible etiologies for nondisjunction include maternal and paternal age effect, genetic predisposition, seasonal variation, viral infection, parental irradiation and environmental chemicals [10]. The dNOR variants have been reported to be more frequent among parents of children with trisomy 21. These variants have been proposed as a risk factor for nondisjunction of acrocentric chromosomes [5].

Advanced maternal age is still the only established risk factor known to be involved in the etiology of DS [11]. In our study, the mean maternal age was $31 \pm 6$ years, with 32% of mothers aged 35 years or older. Several investigators have reported a higher maternal age in regular trisomy, emphasizing the positive role of advanced maternal age on nondisjunction [12,13]. The frequency of maternal nondisjunction has been estimated to be 90%–95% (75% meiosis I, 25% meiosis II). Studying the effect of parental age on these subgroups supported an association between advanced maternal age and both meiosis I and meiosis II errors. The association with meiosis II suggests there is at least one maternal age-related mechanism acting around the time of conception [14,15]. Mitotic errors have not been found to be associated with advanced maternal age [16]. Paternal nondisjunction has been reported to account for approximately 5% of cases, with the errors being predominately in meiosis II [17]. In the present study, the mean paternal age was $35.4 \pm 6.29$ years, with 8% of fathers 45 years or older. Most studies have not shown any association between advanced paternal age and the frequency of trisomy 21 [18].

The modal number of Ag-positive NORs in the parents of the DS patients ranged from 7 to 10, with an average of 8.06, compared with 7.65 in the control group — a statistically significant difference. This accords with Leal-Garza et al.
[19], who reported a significantly increased frequency of Ag-positive NORs in parents of DS children. In our DS patients, the modal number of Ag-positive NORs showed a significantly higher value than the controls, possibly due to the effect of the supernumerary 21 chromosome. A significant difference in the size of Ag-stained NORs was found in the parents of the DS patients compared with controls. This is concurs with Spinner et al. [20], who observed larger sizes of Ag-positive NORs for parents of trisomy 21 children than for the controls.

As one-third of all trisomies observed in spontaneous abortions and live births involve the acrocentric chromosomes, it has been suggested that the presence and/or variant of NORs on the short arm of all five acrocentric chromosome pairs predisposes them to nondisjunction [27]. In our study the frequency of dNOR variants among parents of DS patients (16%) was statistically significant compared with the control group. This supports the idea of a causal role for the dNORs in nondisjunction, also supported by Jackson-Cooke et al. [5], who concluded that dNORs are important in the etiology of trisomy 21. They also suggested the presence of the variant might increase the risk of producing a DS child by as much as 20-fold. The frequency of dNOR variants was significantly higher among DS patients (12%) than the controls (1.2%). Again, this concurs with Jackson-Cook et al. [5], who suggested a dNOR variant chromosome might be present in a parent and contribute to nondisjunction, but might not be transmitted to offspring. On the other hand, Schwartz et al. [22] found a higher frequency of dNOR variants in parents of trisomy 21 children (20%) than in the control group (14%), but the difference was not statistically significant.

The present study showed the mean maternal and paternal ages for dNOR(+) couples to be significantly lower than for the dNOR(−) couples, in contrast to the study by Jackson-Cooke et al. [5]. A family history of DS was found in one case — a third-degree relative of the mother. The mother was a carrier for a dNOR variant. There are reports of recurrence of trisomy 21 in other family members [23]. There was a statistically significant increase in the frequency of first trimester spontaneous abortion for dNOR(+) couples compared to dNOR(−) couples and this agrees with Jackson-Cook et al. [5]. Unfortunately the chromosomal constitution was not known in any of the spontaneous abortus so a direct comparison of the role of aneuploid-to-euploid conceptuses between dNOR(+) and dNOR(−) couples could not be made.

**Conclusion**

The short arms of human acrocentric chromosomes are the sites of heteromorphism, detectable by conventional staining and various banding techniques. Heteromorphisms of these regions include variations in band pattern and duplication of NORs (dNORs). The present study provides support for the causal role of dNOR in nondisjunction involving chromosome 21. Analysis of NOR heteromorphism can be of value in detecting dNOR carriers with an increased risk of producing DS offspring, and should permit more accurate genetic counselling for families with a history of DS. Further cytogenetic studies should be carried out to establish the pathogenetic role of dNOR variants in nondisjunction of chromosome 21, other acrocentric chromosomes and sex chromosomes.
References


19. Leal-Garza CH et al. Actividad de genes ribosomales y asociacion de cromo-
Down syndrome — establishment of a regional research prize foundation

The Regional Committee, having reviewed the Regional Director’s report on Down syndrome, and recalling its endorsement of the Regional Consultative Committee’s proposal to agree to the establishment of prizes for research on priority problems in the Region, taking into consideration the high incidence of Down syndrome in countries of the Eastern Mediterranean Region:

1. Proposes the establishment of a regional prize for research into Down syndrome;
2. Conveys its appreciation to Dr Adbdel Rahman Abdulla Al-Awadi for the generous offer of funds to be used to initiate this prize;
3. Approves the text of the statutes of the Down Syndrome Research Prize Foundation in the Eastern Mediterranean Region;
4. Recommends the establishment of such a foundation to the 103rd session of the Executive Board to be held in January 1999;
5. Invites Member States, institutions and individuals to make voluntary financial contributions to WHO in order to increase the funds available to the foundation for the award of such a prize.